Dehydroascorbate Reductase

Microplate Assay Kit

User Manual

Catalog # CAK1054

(Version 1.2C)

Detection and Quantification of Dehydroascorbate Reductase (DHAR) Activity in Tissue extracts, Cell lysate Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION.....2
II. KIT COMPONENTS.....3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....3
IV. SAMPLE PREPARATION.....4
V. ASSAY PROCEDURE.....5
VI. CALCULATION.....6
VII. TECHNICAL SUPPORT.....7
VIII. NOTES.....7

I. INTRODUCTION

Plant dehydroascorbate reductase (DHAR) is a physiologically important reducing enzyme in the ascorbateglutathione recycling reaction. DHA must be converted to AsA by DHAR in the presence of glutathione (GSH) as a reducing agent. Thus, DHAR is a key factor in maintaining a reduced AsA level in the adaptation to environmental conditions.

The assay is initiated with the enzymatic catalysis of the GSH by DHAR. The enzyme catalysed reaction products AsA can be measured at a colorimetric readout at 265 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well UV Microplate	1 plate	
Assay Buffer	30ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Substrate I	Powder x 1	-20 °C, keep in dark
Substrate II	Powder x 1	-20 °C, keep in dark
Technical Manual	1 Manual	

Note:

Substrate I: add 1 ml Reaction Buffer to dissolve before use.

Substrate II: add 1 ml Reaction Buffer to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 265 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 16000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 16000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Warm the Reaction Buffer to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Blank
Reaction Buffer	160 µl	160 µl
Substrate I	10 µl	10 µl
Substrate II	10 µl	10 µl
Distilled water	--	20 µl
Sample	20 µl	--
Mix, measured at 265 nm and record the absorbance of 10 seconds and 130 seconds.		

1) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of DHAR is the amount of enzyme that generates 1 μmol AsA per minute.

1. According to the protein concentration of sample

$$DHAR\ (U/mg) = [(OD_{Sample(130S)} - OD_{Sample(10S)}) - (OD_{Blank(130S)} - OD_{Blank\ (10S)})] / (\varepsilon \times d) \times V_{Total} / (V_{Sample} \times C_{Protein}) / T$$

$$= 0.153 \times [(OD_{Sample(130S)} - OD_{Sample(10S)}) - (OD_{Blank(130S)} - OD_{Blank\ (10S)})] / C_{Protein}$$

2. According to the weight of sample

$$DHAR\ (U/g) = [(OD_{Sample(130S)} - OD_{Sample(10S)}) - (OD_{Blank(130S)} - OD_{Blank\ (10S)})] / (\varepsilon \times d) \times V_{Total} / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 0.153 \times [(OD_{Sample(130S)} - OD_{Sample(10S)}) - (OD_{Blank(130S)} - OD_{Blank\ (10S)})] / W$$

3. According to the quantity of cells or bacteria

$$DHAR\ (U/10^4) = [(OD_{Sample(130S)} - OD_{Sample(10S)}) - (OD_{Blank(130S)} - OD_{Blank\ (10S)})] / (\varepsilon \times d) \times V_{Total} / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 0.153 \times [(OD_{Sample(130S)} - OD_{Sample(10S)}) - (OD_{Blank(130S)} - OD_{Blank\ (10S)})]/ N$$

ε: molar extinction coefficient, 5.42×10^4 L/mol/cm = 54.2 ml/μmol/cm;

d: the optical path of 96-Well microplate, 0.6 cm;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

V_{Total}: the total volume of the enzymatic reaction, 0.2 ml;

V_{Sample}: the volume of sample, 0.02 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 2 minutes.

VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

VIII. NOTES